RESEARCH PAPERS
FACULTY OF MATERIALS SCIENCE AND TECHNOLOGY IN TRNAVA
SLOVAK UNIVERSITY OF TECHNOLOGY IN BRATISLAVA

10.1515/rput-2017-0008 2017, Volume 25, Number 40

STUDY OF WASTEWATERS CONTAMINATED WITH HEAVY METALS IN BIOETHANOL PRODUCTION

Alica BARTOŠOVÁ, Lenka BLINOVÁ

SLOVAK UNIVERSITY OF TECHNOLOGY IN BRATISLAVA,
FACULTY OF MATERIALS SCIENCE AND TECHNOLOGY IN TRNAVA,
INSTITUTE OF INTEGRATED SAFETY,
ULICA JÁNA BOTTU 2781/25, 917 24 TRNAVA, SLOVAK REPUBLIC
e-mail: alica.bartosova @stuba.sk, lenka.blinova@stuba.sk

Abstract

Bioethanol as a substitute for traditional sources of energy, especially oil transport, is currently one of the most researched alternative motor fuels. Normally, bioethanol is produced from agricultural crops such as sugar cane or corn. However, this is counter-productive, because agriculture is primarily serving to ensure enough food for the people. It is therefore necessary to look for new production of appropriate non-food crops or find an added value to this process. Utilisation of contaminated water from metal industry could be one of them. Based on the hypothesis of reduction of some toxic metals with higher oxidation number is opening the possibility of using this wastewater in alcohol fermentation of any kind of biomass. In this study, hexavalent chromium Cr(VI) was used as a model contaminant in the process of aerobic fermentation of corn to bioethanol. To determine the reduction potential of glucose to Cr(VI), and to quantitatively determinate the glucose content after saccharification, UV/VIS spectrophotometry was used. As a method of qualitative determination of fermentation product, gas chromatography with mass detection was used. Infrared spectrometry was used for qualitative analyses of produced ethanol. Based on the established results shown in this paper, we can conclude that the presence of hexavalent chromium in the fermentation process does not have a significant negative impact, while offering the opportunity of using the industrial wastewaters for the production of bioethanol fuel.

Key words

bioethanol, wastewater, heavy metals, hexavalent chromium, reduction

INTRODUCTION

Heavy metals are naturally occurring elements, but their multiple industrial, domestic, agricultural, medical, and technological applications have led to their wide distribution in the environment. Because of their high degree of toxicity, arsenic, cadmium, chromium, lead, and mercury rank among the priority metals that are of the public health significance. These metallic elements are considered systemic toxicants that are known to induce multiple organ damage, even at lower levels of exposure (1). The presence of heavy metals in aquatic systems has become a serious threat and has a great potential to cause environmental-derived cancer because these metals are non-biodegradable, and therefore persistent (2). Metals are mobilized and carried into the food web as a result of leaching from waste dumps, polluted soils and water (3).

Discharge of untreated or poorly treated wastewater containing toxic heavy metals from industrial effluents into the natural water bodies is a major environmental problem because of their high toxicity and their tendency to accumulate through the food chain (4). These metals increase in concentration at every level of the food chain, and are passed onto the next higher level in a phenomenon called biomagnification (3).

The major disadvantage of conventional treatment technologies is the production of toxic chemical sludge, whose disposal/treatment becomes a costly affair and is not environment-friendly. Therefore, removal of toxic heavy metals to an environmentally safe level in a cost-effective and environmentally friendly manner is of great importance. The conventional technique for metal remediation includes common physical-chemical precipitation, such as inducing electrochemical treatment, chemical coagulation, reverse osmosis, ion exchange and ultrafiltration. In recent years, considerable attention has been devoted to the study of the removal of heavy metal ions from solution by adsorption using agricultural materials. Natural materials that are available in large quantities or certain wastes from agricultural operations may have the potential of being used as low-cost adsorbents, representing unused resources, which are widely available and are environmentally friendly. Some investigations on the removal of heavy metal ions with agricultural by-products have been previously reported. Algae, fungi, bacteria, parts of some higher plants and yeasts have proven to be potential metal biosorbents (3).

The fact that some toxic metals with higher oxidation number can be reduced to lower oxidation number opens new possibilities of using this waste water also in alcohol fermentation. During the process of bio-mineralization, the action of microorganisms transformed metal ions from aqueous medium in the amorphous or crystalline precipitates. In addition, the presence of metals such as zinc or magnesium can significantly affect the fermentation process, because they act as catalysts for glycolytic enzymes and yeasts.

Depending on its oxidation state, of chromium, chromium can have different biological effects, with Cr(VI) that is highly toxic to most of organisms, and Cr(III) that is relatively innocuous (5).

The studies (6) and (5) confirmed the ability of Cr(VI) to be reduced in the process of fermentation to Cr(III).

The mechanisms of chromium tolerance or resistance of selected microbes are of particular importance in both bioremediation and waste water treatment technologies. Studies of various microbial uptake mechanisms for heavy metals could result in the identification of specific microbes with promising potential for application in metal bioremediation (7).

The main objective of this article is to study the presence of hexavalent chromium in the process of fermentation of bioethanol production with regard to the use of heavy metal contaminated waste water in the process of production of alcoholic biofuels.

MATERIALS AND METHODOLOGY

Study of reduction potential of glucose to hexavalent chromium

To confirm the ability of glucose to reduce Cr(VI) (stock solution was prepared from $K_2Cr_2O_7$), a series of experiments were performed. Glucose solutions with concentration of 5, 7 and 10 g/L were mixed with chromium solutions in concentration of Cr(VI) 5; 7 and 10 mg/L and let to react for 2 minutes. Reduction reaction equation can be written as follow:

$$3R - COH + Cr_2O_7^{2-} + 8H^+ \rightarrow 3RCOOH + 2Cr^{3+} + 4H_2O.$$

In the samples, Cr(VI) was determined by 1,5-diphenylcarbazide spectrophotometric method (UV/VIS Spectrophotometer GENESYS™). The reaction of Cr(VI) with 1,5-diphenylcarbazide is very sensitive and highly selective, and therefore especially suitable for the detection and determination of very small amounts of Cr(VI). The formation of complex is easier in acid solution. Mixture of test solution with sulphuric acid and reagent solution forms a pink coloured complex, which can be measured by spectrophotometric method at 540 nm[8]. Each sample was measured in triplicate.

Enzymatic saccharification

In enzymatic hydrolysis, starch is converted to glucose by enzymes. This process can be written as follows:

$$(C_6H_{10}O_5)_n + n(H_2O) \xrightarrow{enzyme} n(C_6H_{12}O_6).$$

For two-step enzymatic hydrolysis, about 175 g of dry milled corn (Oxxygen, Grange Hronské Kľačany) was mixed with 325 mL of distilled water. For the Cr(VI) addition, the solutions in concentration of 5, 7 and 10 mg/L were prepared. Nitric acid was added to adjust pH to 5 – 5.3. The mixture was then supplemented with 7 mL α-amylase (Termamyl EC 3.2.1.1, Novozymes DE), and incubated at 85 °C (9). The process of liquefaction lasted for 120 minutes. Then, the samples were cooled down to 60 °C and reducing sugars were determined (10).

In the second step of hydrolysis – saccharification, pH was adjusted to 4.5, and 20 mL amyloglucosidase (Saczyme EC 3.2.1.3, Novozymes DE) were added. The mixture was incubating at 60 – 63 °C for 16 hours. Experiment was made in triplicate.

Ethanol fermentation

Each ethanol fermentation was carried out in 1 L glass bioreactor (glass bottle with fermentation cup + stirrer RW 16 KA, pH measuring transducer GPHU 014MP). The amount of 10 mL of inoculated medium (4 g *Saccharomyses cerevisiae* dissolved in 5% w/w solution of glucose, incubated 10 min at temperature 30 °C) was added to the hydrolysate mixtures. After careful stirring, the fermentation cups were closed and process of fermentation took approximately 70 hours at 30 °C temperature.

Composition determination of fermentation solutions by Gas chromatography – Mass Spectroscopy

The fermentation solutions were subjected to GC-MS analyses on the instrument of Gas chromatograph Agilent 5975C with Mass Detector with capillary column (Agilent Technologies HP – 5ms, 5% fenyl-95% methylsiloxan, 30 m x 0.250 mm ID x 25 μm) and GC-MS 3975C inert XL Data Analyzer software. Initially the oven temperature was maintained at 40 °C for 5minutes, and then the temperature was gradually increased to 260 °C per 40 °C/min at a rate of held for 3°C/min. Injection was set at split mode with split ratio 10:1. Helium gas 99.999% of purity was used as a carrier gas. The flow rate of helium gas was set to 0.68 mL/min with pressure set at 20684 Pa and average velocity 30 cm per second.

Analysis of produced bioethanol by Infrared spectroscopy with Fourier Transformation

For qualitative analysis of produced bioethanol infrared spectroscopy with Fourier transformation with ATR technique - Attenuated Total Reflectance (Varian 660 Dual MidIR MCT/ TGS Bundle) was used. Samples of ethanol were directly applied to a diamante crystal of ATR and the resulting spectra were corrected for the background air absorbance. The spectra were recorded using Varian Resolutions Pro, and samples were measured in the region of 4000 - 400 cm^{-1}; each spectrum was measured 256 times, at resolution 4. To minimize differences which were due to baseline shifts, the spectra were baseline corrected and ATR-corrected.

RESULTS AND DISCUSSION

Study of reduction potential of glucose to hexavalent chromium

As can be seen in Fig.1, glucose has ability to reduce the concentration of hexavalent chromium. For example, the initial chromium Cr(VI) concentration 10 mg/L in response to glucose solution with a concentration of 5 g/L caused a decrease of 2.6 mg/L, representing a decrease of approximately 27.5%.

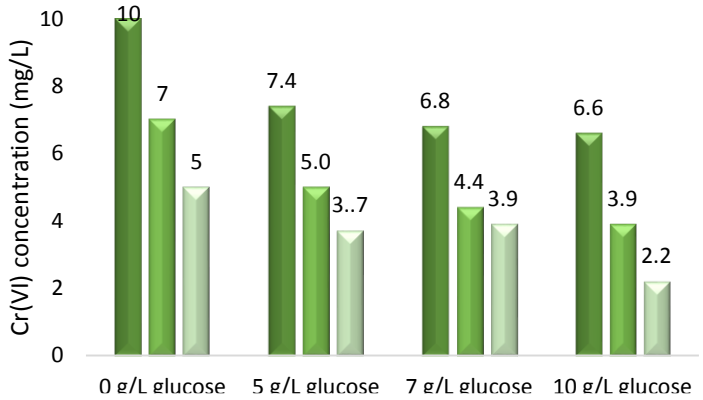

Fig. 1 *Comparison of reducing effects of different concentrations of glucose to Cr(VI)*

The most pronounced decrease was recorded at the initial concentration of 5 mg/L on exposure to glucose solution at a concentration of 10 g/L to reduce the amount of hexavalent chromium to 2.2 mg/L, thus about 56%. These results show ability of glucose to reduce Cr(VI) in short time.

Saccharification and fermentation of corn starch with and without addition of Cr(VI)

As can be seen from the Fig. 2, the presence of hexavalent chromium had a positive effect on enzymatic hydrolysis of corn starch only to a certain concentration. Compared to

the samples without Cr(VI) and samples with concentration of 5 mg/L Cr(Vl), dextrose equivalent increased by approximately 3%.

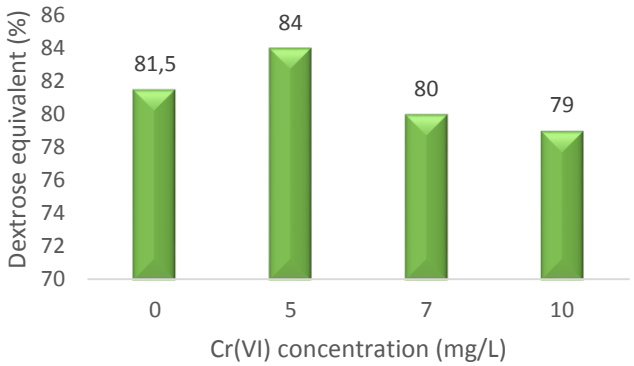

Fig. 2 *Dextrose equivalent of corn starch samples with different concentrations of Cr(VI) after saccharification*

At higher concentrations, the enzyme activity was reduced due to the lower glucose production and hence a decrease in dextrose equivalent DE of 1.98% for a sample at a concentration of 7 mg/L and 2.5% for the concentration of 10 mg/L. The fact that higher concentrations caused a decrease in the enzyme activity and, vice versa, lower concentration increases activity is confirmed by the study (11) which investigated the effect of Cr(VI) on the activity of amylase in barley substrate.

The next step was the actual fermentation by the yeast *Saccharomyces cerevisiae*, which was carried out for about 62 hours for fermentation with solutions of Cr(VI), and 70 hours for fermentation without addition of chromium. This decrease of the fermentation time is probably attributable to a reducing effect of glucose Cr(VI) when this is reduced to Cr(III), which consumes a significant amount of glucose. Loss of glucose throughout the duration of the fermentation can be expressed by the decrease of reduced sugars (Fig. 3).

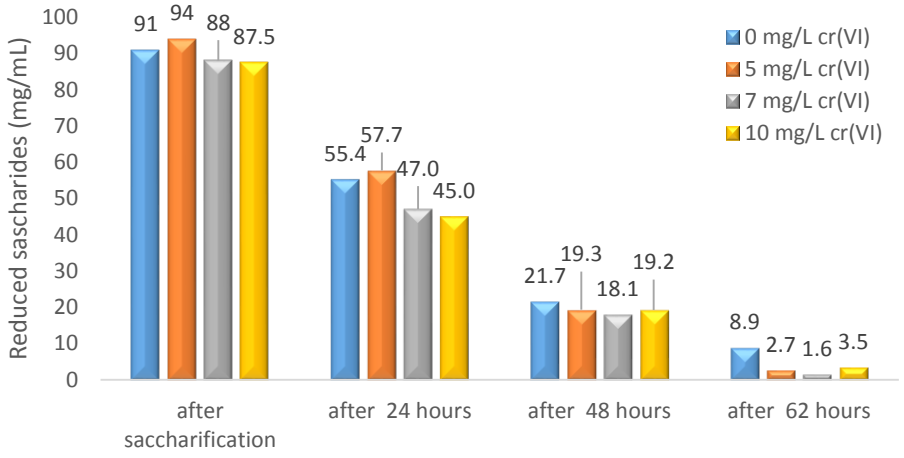

Fig. 3 *Reducing sugars during fermentation with various concentrations of Cr(VI)*

As could be seen from Fig. 3, the content of the reducing sugars in samples with increasing concentration of Cr(VI) decreased after the very first day. The largest fall was recorded in the sample with the highest concentration of 10 mg/L; it presents 42.9 mg/mL, which was caused both by converting glucose to ethanol but also its depletion to reduce hexavalent chromium to trivalent. Simultaneously, turbulent formation of CO_2 was observed. After 48 hours approximately more than half of glucose content was consumed in all samples of fermentation experiments. Subsequently, the amount of non-fermented glucose was determined at the end of the fermentation.

Subsequently, the fermentation slurry was filtered and the sample was removed for qualitative determination of composition of the filtrate by GC-MS. Chromatographic records of filtrates are shown in Fig. 4.

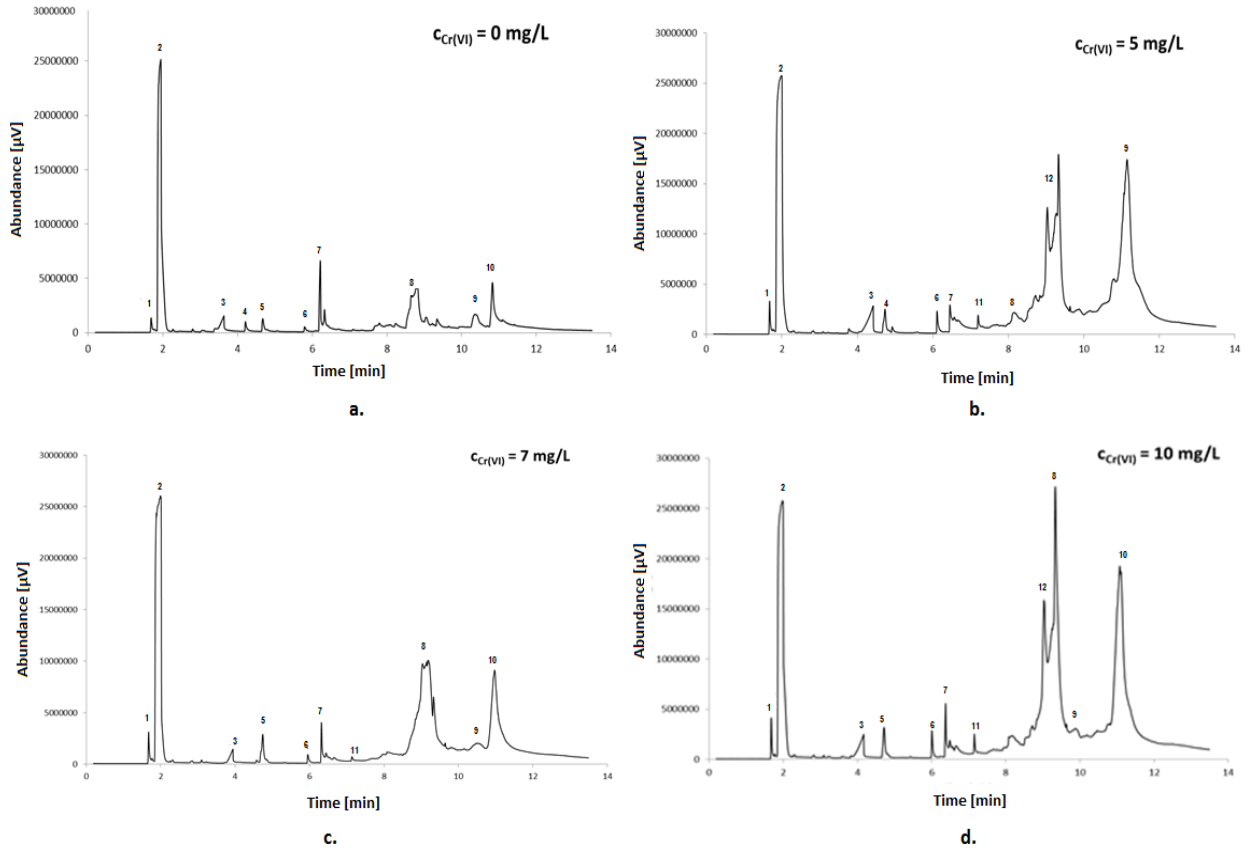

Fig. 4 *Chromatograms of filtrate after fermentation (a.) solution without present of Cr(CV), (b.) solution with concentration of Cr(CV) 5 mg/L, (c.) solution with concentration of Cr(CV) 7 mg/L, (d.) solution with concentration of Cr(CV) 10 mg/L*

All filtrates from the fermentation of corn starch contained the ethanol (2) and residual amounts of carbon dioxide (1). The presence of a small amount of acetic acid (3) is due to the natural biological oxidation of ethanol and the action of yeast is also produces with a well-managed fermentation. Further, alcohols such as propanol (4) and butanol (5) were recorded as well as glycerol (8), which is also a by-product of the fermentation of starch into ethanol, as well as acrylic acid (6). Residues of saczyme enzyme of glucopyranose (9) and glucopiranoside (10) are found here in small amounts.

In addition to the ethanol in the sample (b.) of the filtrate after fermentation with a concentration of Cr(VI) 5 mg/L, also other typical by-products of fermentation were located. The biggest difference in the compared samples with and without the presence of Cr(VI) can be observed during the time period from 9.04 to 9.34 minutes, where the double

peak identified as pyranone (12), that was not found in the sample without the addition of Cr(VI), occurred, On the other hand, furfural (11) is presented just in the samples with Cr(VI). The last significant peak with a retention time of 11.16 minutes was attributed to the rest of ethyl α-d-glucopyranoside (10). Similar results were also observed in the chromatogram trace of the sample with the concentration of Cr(VI) 7 mg/L (c.)

Table 1 shows all the detected compounds originated in studied fermentation processes with/without presence of hexavalent chromium. Concentration of individual products has not been determined.

Table 1 List of compounds originated in fermentation process with various concentrations of hexavalent chromium

Compound		Formula	Retention time [min]			
			Cr(VI) 0 mg/L	Cr(VI) 5 mg/L	Cr(VI) 7 mg/L	Cr(VI) 10 mg/L
1	**Carbon dioxide**	CO_2	✓	✓	✓	✓
2	**Ethanol**	C_2H_6O	✓	✓	✓	✓
3	**Acetic acid**	$C_2H_4O_2$	✓	✓	✓	✓
4	**2-Propanone, 1-hydroxy-**	$C_3H_6O_2$	✓	none	none	none
5	**3-methyl-,1-Butanol**	$C_5H_{12}O$	✓	✓	✓	✓
6	**Acrylic acid**	$C_6H_8O_3$	✓	✓	✓	✓
7	**2,3-Butanediol**	$C_4H_{10}O_2$	✓	✓	✓	✓
8	**Glycerol**	$C_3H_8O_3$	✓	✓	✓	✓
9	**β-D-Glucopyranose**	$C_{12}H_{22}O_{11}$	✓	✓	none	✓
10	**Ethyl α-d-Glucopyranoside**	$C_8H_{16}O_6$	✓	none	✓	✓
11	**Furfural**	$C_5H_6O_2$	none	✓	✓	✓
12	**Pyranone**	$C_6H_8O_4$	none	✓	none	✓

Qualitative analysis of produced bioethanol

After the second distillation, samples were taken to determination of quality of bioethanol using FTIR. Spectrograms of bioethanol samples with and without the presence of Cr(VI) obtained after distillation of fermentation slurry can be seen in Fig. 5. Area of fingerprint region typical for ethanol is in wavenumber range from 800 to 1200 cm^{-1}. Peaks at 1043 cm^{-1} and 1085 cm^{-1} are attributed to C-O stretching bond. Peak at 1452 cm^{-1} indicates the presence of C-O-H deformation bond. The O-H bond in alcohols absorbs IR irradiation at wavenumbers of about 3230 – 3550 cm^{-1}, which, in this case, is at 3331 cm^{-1}. Also, typical peak area for ethanol 2800 – 3000 cm^{-1} is assigned to C-H bonds. Similar spectra of ethanol were measured also in (12).

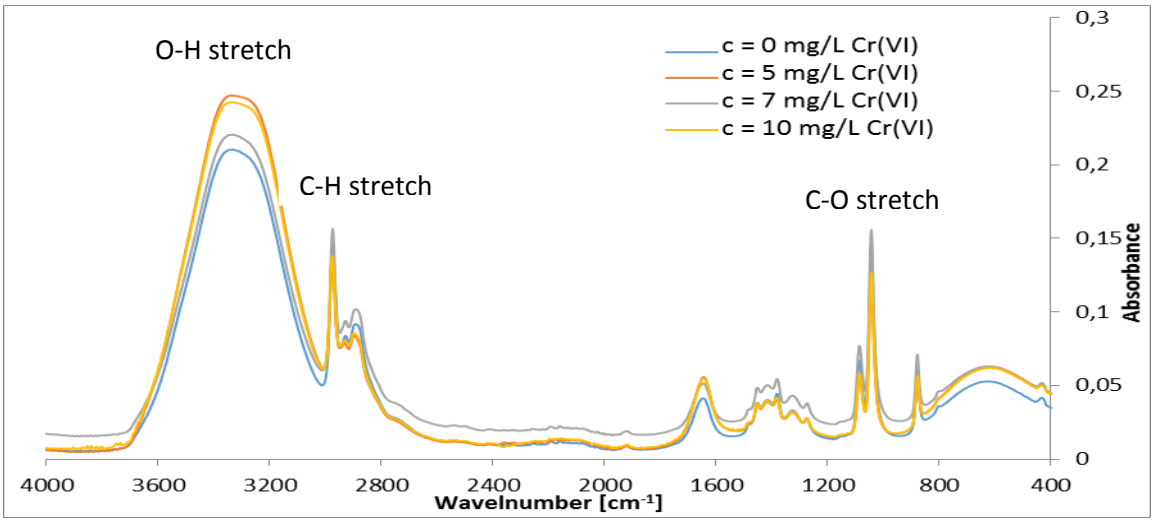

Fig. 5 *IR spectrums of bioethanol samples*

CONCLUSIONS

The objective of this paper was to study present hexavalent chromium in alcoholic fermentation with regard to the use of contaminated waste waters by toxic metals in bioethanol production.

Several conclusions can be drawn from the obtained results:

- In the process of enzymatic hydrolysis, a lower concentration of hexavalent chromium (5 mg/L) did not have a significant effect (DE was 3 % higher than in the sample without presence of Cr(VI)).
- Duration of fermentation was shortened – it is because some of the glucose was reduced for Cr(VI) as well as for the bioethanol production. Fermentation in presence of Cr(VI) was eight hours shorter. However, the amount of produced ethanol was not measured, because the samples were taken for the process analyses. It affected the total volume of produced bioethanol.
- The presence of Cr(VI) in the process of alcoholic fermentation had no significant effect; approximately the same by-products were detected in all samples. The difference in compared samples with and without the presence of Cr(VI) can be observed during the period from 9.04 to 9.34 minutes, where the double peak identified as pyranone that was not found in the sample without the addition of Cr(VI).
- Analysis of produced bioethanol was performed by FTIR. After distillation of fermentation slurry, quality of bioethanol was same in all samples.

Usage of waste water with hexavalent chromium in process of fermentation of conventional biomass could be an effective way how to dispose toxic waste water as well as produce biofuel as bioethanol.

Acknowledgement

This article was written with the support of the project incubator of new ideas No. 1369 (2016): *Study of synthesis subsequent use of different types of nanocomposites*.

References:

1. TCHOUNWOU, P. B., YEDJOU, C. G., PATLOLLA, A. K., SUTTON, D. J. 2012. Heavy Metals Toxicity and the Environment. *EXS*, Vol. **101**, pp. 133–164.
2. AGRAFIOTI, E., KALDERIS, D., DIAMADOPOULOS, E. 2014. Arsenic and chromium removal from water using biochars derived from rice husk, organic solid wastes and sewage sludge. *J. Environ. Manage.*, Vol. **133**, pp. 309–314.
3. AL-QAHTANI, K. M. 2016. Water purification using different waste fruit cortexes for the removal of heavy metals. *J. Taibah Univ. Sci.*, **10**(5), pp. 700–708.
4. Dehghani, M. H., Sanaei, D., Ali, I., Bhatnagar, A. 2016. Removal of chromium(VI) from aqueous solution using treated waste newspaper as a low-cost adsorbent: Kinetic modeling and isotherm studies. *J. Mol. Liq.*, vol. **215**, pp. 671–679.
5. FOCARDI, S., PEPI, M., FOCARDI, S. E. 2013. Microbial Reduction of Hexavalent Chromium as a Mechanism of Detoxification and Possible Bioremediation Applications. *Biodegrad. - Life Sci.*, No. Iii.
6. LEITA, L., MARGON, A., SINICCO, T., MONDINI, C. 2011. Glucose promotes the reduction of hexavalent chromium in soil. *Geoderma*, **164**(3–4), pp. 122–127.
7. SHUGABA, A., BUBA. F. 2012. Uptake and Reduction of Hexavalent Chromium by Aspergillus Niger and Aspergillus Parasiticus. *J. Pet. Environ. Biotechnol.*, **03**(03).
8. EPA method 7196A *Chromium, Hexavalent (Colorimetric)*, 1992.
9. YAO, L., LEE, S. L., WANG, T., DE MOURA, J. M. L. N., JOHNSON, L. A. 2012. Effects of fermentation substrate conditions on corn-soy co-fermentation for fuel ethanol production. *Bioresour. Technol.*, Vol. **120**, pp. 140–148.
10. MILLER, G. L. 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Anal. Chem.*, vol. **3**, pp. 426–428.
11. OLTEANU, Z., OPRICA, L., TRUTA, E., ZAMFIRACHE, M., ROSU, M. C. 2012. *Changes induced by two chromium-containing compounds in antioxidaive response, soluble protein level and amylase activity in barley seedlings*.
12. NAGARAJAN, R., MEHROTA, R., BAJAJ, M. M. 2006. Quantitative analysis of methanol, an adulterant in alcoholic beverages, using attenualed total reflectance spectroscopy. *Journal of Scientific and Industrial Research*, Vol. **65**, pp. 416 – 419. ISSN:0975-1084

ORCID:

Alica Bartošová 0000-0003-1442-9652
Lenka Blinová 0000-0001-6971-6558